

## Clinicopathological factors breast cancer recurrence and the effect of molecular subtypes

Recurrence in breast cancer

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### Abstract

**Aim:** The prognostic and predictive factors for breast cancer are well defined today. However, there is still no information available to help us determine possible recurrence localizations. Our study aimed to examine the clinicopathological variables that affect metastatic behavior of breast cancer and its relationship with molecular subtypes.

**Material and Methods:** Two hundred patients with breast cancer operated in a surgical oncology clinic were included in our study. Clinicopathological and demographic characteristics were recorded retrospectively from the hospital database. The patients were categorized per the TNM staging system. According to the immunohistochemical results, the subtypes were defined as luminal A, luminal B/human epidermal growth factor receptor 2+(HER2+), luminal B/HER2-, HER2-rich, and triple-negative (TNBC). Survival analyzes were estimated using the Kaplan-Meier method. Variables that were statistically significant in the univariate analysis were then included in the multivariate analysis using the Cox proportional hazards regression model. The relationship between categorical variables was analyzed using the chi-square ( $\chi^2$ ) test. Statistical analysis was made at a 95% confidence interval. A p-value higher than 0.05 was considered statistically significant.

**Results:** The most common recurrence was observed in bone tissue (59%). A significant correlation was found between recurrence localizations and molecular subtypes ( $p=0.025$ ). Luminal subtypes were mostly related to bones, and non-luminal subtypes were mostly associated with visceral and brain metastases. Approximately 1/3 of the metastases were in the form of multiorgan involvement. Factors affecting tumor recurrence were tumor size ( $p=0.029$ ), axillary lymph node involvement ( $p=0.047$ ), LVI status ( $p=0.018$ ), histological grade of the tumor ( $p=0.028$ ), TNM stage ( $p=0.035$ ), and local stage ( $p=0.019$ ).

**Discussion:** The new clinical/diagnostic staging system, including molecular subtypes, can enable us to better predict the probability of distant recurrence and their possible localization.

### Keywords

Breast Cancer; Clinicopathological Characteristics; Luminal Subtype; Recurrence

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## Introduction

Despite modern strategies for follow-up and treatment, up to 30% of patients with postoperative breast cancer (BC) develop distant disease recurrence. However, we still do not have comprehensive information about tumor spread and regional recurrence patterns [1]. Survival has improved significantly as a result of early diagnosis and treatment. Also, the number of women who need follow-up after treatment has increased significantly. The primary purpose of follow-up includes early detection of distant and near recurrences and second primary tumors. The incidence of recurrence after initial treatment is affected by prognostic factors such as age, histological grade, axillary lymphatic involvement, hormone receptor status, vascular invasion, and initial treatment status [2, 3].

In parallel with the increasing understanding of cancer biology, significant advances have been made in treatment. Genomic studies have shown that breast cancer is not a single disease, but a heterogeneous group of diseases. These studies will guide us in determining the personalized treatment profile. There are staging systems to guide us in determining our follow-up and treatment strategies. The TNM staging system is a projection of the clinicopathological status based on key parameters such as tumor size, node state, and metastatic disease state. However, studies have shown that the metastatic character of the tumor also carries genomic signatures. This heterogeneous nature of breast cancer also explains the difficulty in predicting the progression of the disease. The gene profile expresses itself at receptor levels as detected by immunohistochemical (IHC) staining. Accordingly, breast cancer is divided into five intrinsic subgroups. These are predominantly Luminal-like subtypes (Luminal) expressing estrogen receptor (ER) and progesterone receptor (PR), basal-like triple-negative subtypes (TNBC) that overexpress HER2 but are rich in HER2 negative for estrogen-progesterone expression and do not predominantly express ER, PR, or HER2 receptors. However, for comprehensive characterization, to reveal the full heterogeneity of breast cancer, all genome profiling that is not routinely used is needed [4].

In this study, we aimed to examine the relationship between molecular subtypes and the TNM stage to help us understand the spread pattern in patients with breast cancer who were operated on but recurred. Recognition and appreciation of these clinically different subgroups of BC can help us predict different outcomes and provide new insights into disease management. With the increasing understanding of tumor biology, it is hoped that ongoing and future clinical trials will transform into better outcomes for patients.

## Material and Methods

Our study was initiated with the approval of the Ankara University Faculty of Medicine Hospital Ethics Committee Decision number: İ10-623-20). Written informed consent was obtained from all participants.

Two hundred twenty-one patients operated on for breast cancer in the Surgical Oncology Clinic between 2010 and 2020 were included in the study. Twenty-one patients were excluded due to missing data. The hospital database was analyzed retrospectively. From the pathological examination results

of the patients, ER, PR, and HER2 status, KI-67 percentage, tumor type, size and histological grade, lymphovascular invasion (LVI) status, axillary lymph node involvement status were recorded. Besides, the patients' age, menopause status, and the type of surgical procedure performed were examined and recorded in the digital patient files. Thirty-nine of the patients had various organ recurrences. Distant recurrence was defined as BC recurrence beyond the margins of the ipsilateral breast, chest wall, or regional lymph nodes. The sites of distant recurrence were categorized as follows: bone, brain (including leptomeninges), liver, lung, distant nodal (including supraclavicular internal mammary nodules other than ipsilateral axillary), and multiple organ recurrence.

We classified patients according to the recommendations of the St. Gallen International Expert Consensus Report (2013) for molecular breast cancer subtypes. The patients were categorized by the receptor status of their primary tumor as follows: Luminal A (ER + and/or PR + and HER2-, Ki-67 < 14%); luminal B /HER2- (ER + and/or PR +, HER2- and Ki-67  $\geq$  14%); luminal B/HER2 + (ER + and/or PR +, HER2+, any Ki-67); HER2-rich (ER- and PR- and HER2 +) and TNBC (ER- and PR- and HER2-) [5]. ER and PR status were determined using immunohistochemical staining (IHC). Tumors were considered HER2-positive only if they showed HER2 amplification (ratio > 2) using 3+ staining with IHC staining or fluorescent in situ hybridization (FISH). Tumors were also classified as Luminal and non-luminal based on hormone receptor expression.

They were staged according to the TNM system based on the American Joint Committee on Cancer (AJCC) 18th Edition (stage 1A,1B,2A,2B,3A,3B,3C,4) [6]. Tumors were also classified by their local stages as local (stages 1, 2) and locally advanced stage (stage3).

## Statistical Analysis

Descriptive statistical analyzes were performed and all data were presented as mean  $\pm$  standard deviation (SD), number, percentage, maximum and minimum values. Survival curves were estimated using the Kaplan-Meier method, and the significance of the differences between these curves was determined using the log-rank test. Variables that were statistically significant in the univariate analysis were then included in the multivariate analysis using the Cox proportional hazards regression model. The relationship between categorical variables was analyzed using the chi-square ( $\chi^2$ ) test. Statistical analysis was done at a 95% confidence interval. A P-value higher than 0.05 was considered statistically significant (all reported p-values were two-tailed).

## Results

All 200 patients included in the study were women. The mean follow-up period was  $104.9 \pm 3.5$  months; 19.5% (n=39) patients had recurrence, while 80.5% (n=161) did not. According to the menopausal status, 45% of the patients (n=90) were premenopausal, 55% (n=110) were post-menopausal; 54% (n=108) of the patients were right breast and 46% (n=92) were left breast patients. The patients' mean age was  $53.5 \pm 5.2$  (28-84) years, and the mean KI-67 percentage was  $28.8 \pm 17.7$ . Half of the patients had no axillary involvement. Mastectomy procedure was performed in approximately half of the patients, and breast-

conserving surgery was performed in the other half. The most common histological type was invasive ductal carcinoma 68% (n=136), followed by lobular carcinoma (19.n=38) and other histological types with decreasing frequency. The mean number of pathological lymph nodes removed was 4.4±3 (1-28), and the mean number of total lymph nodes removed was 12±7.2 (1-35). The distribution of clinicopathological characteristics by groups is presented in Table 1.

**Table 1.** Recurrence-related cox regression analysis results

Characteristics	Metastatic group (39) N(%)	All patients (200) N(%)	OR (ExpB)	95%CI	P-value
T stage			3	1.3-7.2	0.029
T1 (<2 cm)	12(30.8)	96(49)			
T2 (2-5 cm)	18(46.2)	80(40)			
T3 (> 5 cm)	9(23)	22(11)			
Nodal status			1.95	1-3.8	0.047
Negative	13(33)	90(45)			
Positive	26(67)	110(55)			
LVI			2.3	1.13-4.4	0.018
Negative	12(30.8)				
Positive	27(69.2)				
Tumor grade			6.6	1-53	0.028
Grade 1	1(2.6)	32(16)			
Grade 2	17(43.6)	74(37)			
Grade 3	21(53.8)	94(47)			
TNM stage			3.8	1.5-9.8	0.035
Local stage			2.13	1.1-4	0.019
Early stage	24(61.5)	150(75)			
Advanced stage	15(38.5)	50(25)			
Molecular subtype					0.373
Luminal A	6(15.4)	44(22)			
Lum B, HER2-	9(23)	68(34)			
Lum B, HER2+	13(33.2)	54(27)			
HER2+	6(15.4)	16(8)			
TNBC	5(13)	18(9)			

OD: odds ratio; CI: confidence interval; LVI, Lymphovascular invasion

A significant correlation was found in x2 analysis between recurrent regions and molecular subtypes (p=0.025). Bone metastases were mainly associated with luminal subtypes, while visceral metastases were associated with non-luminal subtypes.

The most common metastatic sites were 59% bone, 23% lung, 20.5% liver, 15.4% axilla, 10% distant nodal, and 7.6% brain. One-third of these metastasis sites had multiorgan involvement, and in general, the liver and lungs accompanying bone metastases were in the form of different combinations, albeit a little. All but two of the bone metastases were associated with luminal subtype breast cancer. Internal organ involvement accompanied two non-luminal metastases. While axillary recurrences were equally distributed, 2 of the three patients with brain metastases had TNBC subtype.

The mean follow-up period of patients with recurrence was 58 ±28(1-154) months, respectively 1,2,5,10 years disease-free survival rate (DFS) was (DFS) 96.7%-93.2%-87.3%-72.9%. In the cox regression analysis, factors affecting tumor recurrence were found as tumor size (p=0.029), axillary lymph

node involvement (p=0.047), LVI status (p=0.018), histological grade of the tumor (p=0.028), TNM stage (p=0.035), local stage (p=0.019). However, there was no significant relationship between menopausal status, tumor histology, receptor status, type of surgery and axillary involvement (P > 0.05)

**Discussion**

Our study examined the relationship between recurrence sites, molecular subtypes, and other clinicopathological variables after recurrence in patients operated on for breast cancer. Knowing the variables that determine the disease’s natural course helps define different patient groups by the likelihood of relapse due to the disease. The risk of recurrence development was high in tumors with LVI, pathological involvement at the axilla, high histological grade and size, and advanced local stage at the initial diagnosis time. The most common metastasis area was bone, mainly consisting of tumor recurrences with a luminal subtype. Non-luminal subtypes were mostly associated with visceral recurrences. In particular, the TNBC subtype was mostly accompanied by brain metastases.

Our results are in line with the current literature, [7- 9]. In the study conducted by Geurts et al. with 362 recurrence cases, it was reported that recurrences usually occur in the first year after diagnosis and in the form of distant metastasis. They reported young age (<40), tumor size (T2, T3) and high tumor histological grade (Grades 2, 3), axillary positive lymph nodes, multifocality, and a patient not receiving chemotherapy as prognostic factors for first recurrence [10].

A recently published study from Denmark reported 5-year breast cancer recurrence rates of 18% bone and 5% visceral metastasis in a sample of 23,478 breast cancer patients [11]. Except for patients with widespread metastases, there are two main disease patterns in recurrent breast cancer. Patients with ER +/PR + (luminal) tumors tend to develop more bone metastases but no brain metastases. The situation is opposite in patients with ER-/PR-(non-luminal) tumors [12]. Clinically, the most common metastasis sites are organs such as bone, lung, central nervous system, liver [1,13]. In our study, the most common metastasis site was bone (59%), followed by organs such as lung, liver, distant nodal regions, and brain. Multiorgan involvement was present in one-third of the metastases. These involvements were generally in the form of combinations of organ involvement accompanying bone involvement. Locoregional recurrences (15%) were in the form of axillary involvement, except for one patient with local recurrence. In our study, bone metastases were mostly observed in luminal subtype tumors. While locoregional recurrences were evenly distributed, visceral and brain metastases were more common in non-luminal subtypes. In addition to classical prognostic factors, breast cancer types are closely associated with the risk of recurrence and outcomes, and its prognostic value is widely recognized and guides clinicians [14-17]

Breast cancer recurrence patterns describe a complex interaction of seed and soil factors, including tumor circulation, proliferation, angiogenesis, and the target tissue’s microenvironment. Some relationships between major molecular subtypes and propagation patterns have been identified. HER2 and ER expression status have been associated

with an increased risk of lung and bone metastases. Overall, TNBC has a worse prognosis than other genotypes with the same stage. Metastatic TNBC is accompanied by significantly greater visceral involvement compared to other breast cancers, associated with a dramatic increase in the risk of lung and CNS recurrence [18].

In autopsies performed in patients with breast cancer, the most common cause of death was various organ metastases, accounting for 42% of all deaths. Interestingly, in this study involving 166 cadavers, involvement was observed in unexpected areas. Although these areas included endocrine organs (40%), the lungs (28%), cardiovascular system (21%), and genitourinary system (21%), metastases to bones (10%) and CNS (14%) were very rare [19]. These results show that the detected metastases represent the tip of the iceberg. In reality, the frequency of metastasis is much higher than it appears.

Breast cancer is the second most common cause of CNS metastases. There is a common belief that tumors with lobular histology tend to metastasize to leptomeningeal areas [13]. Two of our three patients who showed brain metastasis had lobular histology.

A new clinical/diagnostic staging process, including molecular subtypes, may better predict the likelihood of distant recurrence and their anatomical location. Recognition of different molecular subtypes clinically can help to evaluate distant recurrences and their possible localization.

Besides these studies, machine learning models that include serum biomarkers and hormone receptors can effectively predict breast cancer metastasis at least three months in advance [19]. Studies conducted have shown an excessive fear of recurrence in women patients, which may affect the prognosis. It was reported that understanding the risk of systemic recurrence, especially in patients with a favorable prognosis, will mean risk communication between clinician and patient, better understanding of risk among patients, and improvements in quality of life [20,21].

### Conclusion

In conclusion, data on the long-term risk of breast cancer recurrence from population-based patient samples are insufficient since the vast majority of published studies use selected patient samples, such as hospital-based cohorts. More population-based randomized studies are needed to determine prognostic factors affecting recurrence patterns and their threshold values.

### Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

### Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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### Conflict of interest

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